Interaction of Plasma Gelsolin with G-actin and F-actin in the Presence and Absence of Calcium Ions*

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Plasma gelsolin formed a very tight 1:2 complex with G-actin in the presence of Ca$^{2+}$, but no interaction between gelsolin and G-actin was detected in the presence of excess EGTA. However, the 1:2 complex dissociated into a 1:1 gelsolin:actin complex and monomeric actin when excess EGTA was added. Plasma gelsolin bound tightly to the barbed ends of actin filaments and also severed filaments in the presence of Ca$^{2+}$ and bound weakly to the filament barbed end in the presence of EGTA. The 1:2 gelsolin-actin complex bound to the barbed ends of filaments but did not sever them. By blocking the barbed end of filaments with plasma gelsolin, we determined the critical concentration at the pointed end in 1 mM MgCl$_2$ and 0.2 mM ATP to be 4 $\mu$M. The dissociation rate constant for ADP-G-actin from the pointed end was estimated to be about 0.4 s$^{-1}$ and the association rate constant to be about 5 x $10^4$ M$^{-1}$ s$^{-1}$. Finally, we obtained evidence that plasma gelsolin accelerates but does not bypass the nucleation step and, therefore, that the concentration of gelsolin does not directly determine the concentration of filaments polymerized in its presence. Thus, gelsolin-capped filaments may not provide an absolutely reliable method for determining the rate constant for the association of ATP-G-actin at the pointed end of filaments, but a reasonable estimate would be $1 \times 10^3$ M$^{-1}$ s$^{-1}$ in 1 mM MgCl$_2$ and 0.2 mM ATP.

In addition to actin, all eukaryotic cells contain a number of actin-binding proteins (1–3) that regulate the kinetics and extent of polymerization of the actin and the organization of actin filaments. The most numerous and most studied group of actin-binding proteins are the capping proteins that bind to the barbed ends$^1$ of filaments. The general properties of capping proteins are easily summarized. They inhibit the rate of addition and loss of actin subunits at the end of the filament to which they are bound, and they act as nucleating factors for polymerization which leads to the formation of more filaments of shorter average length than would have been formed in the absence of the capping protein. At least some barbed-end capping proteins also seem to sever filaments when added to F-actin. Whether by the severing mechanism or by their nucleating property, the same filament/length number distribution is expected when the capping proteins are added to F-actin as when they are added to G-actin. Some of the capping proteins are Ca$^{2+}$ sensitive.

In order to understand fully the regulatory roles of capping proteins in cells, it is necessary first to define in much greater detail the mechanisms of their interactions with purified actin. At the same time, a study of the effects of capping proteins on actin polymerization can provide additional insights into the mechanisms of the still incompletely understood polymerization process.

The simplest equation that describes the elongation of actin filaments is

\[ \frac{dF}{dt} = k_a N c_0 - k_d N = k c (c_1 - c_p) \]  

(1)

where $N$ is the concentration of filament ends, $k_a$ and $k_d$ are the association and dissociation rate constants, $c_1$ is the concentration of actin monomer, and $c_p$ (the critical concentration) is $k_a/k_d$. But, the two ends of the F-actin filament are structurally and kinetically different (4); under most conditions the association and dissociation rate constants are larger at the barbed end. Also, because of the continued hydrolysis of ATP by actin, the two ends can also have different critical concentrations (5); generally the critical concentration is lower at the barbed end (1, 6). Therefore, Equation 1 must be modified as follows:

\[ \frac{dF}{dt} = (k_b + k_p) N c_0 - (k_b + k_p) N = k_b N (c_1 - c_p) + k_p N (c_1 - c_p) \]  

(2)

where $b$ and $p$ refer to the barbed and pointed ends, respectively. Furthermore, because hydrolysis of ATP occurs on the actin filament subsequent to the elongation step (7–9), actin filaments contain a short ATP cap at steady state (9), at least at the barbed end, and can have a long stretch of ATP-actin subunits during periods of net elongation (10). At steady state, the ATP cap stabilizes the very much longer ADP-actin core but the ATP cap is lost during depolymerization, thus exposing less stable ADP-actin ends (9). Thus, several different association and dissociation reactions can occur at each end of the actin filament. The major reactions involve the addition and loss of ADP-actin monomers from ADP-actin filaments, the addition and loss of ATP-actin monomers from ATP-capped filaments, and the addition and loss of ATP-actin monomers from filaments with long stretches of ATP-actin at their ends (10).

Studies of the polymerization of actin alone in bulk solution have provided reasonable estimates of the sums of the rate constants for these six reactions as they occur at both ends of the actin filaments (11). These values will be approximate estimates of most of the rate constants for the barbed ends because the rate constants at the barbed end are generally

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1 The two ends of the polar actin filaments are designated as barbed and pointed because of the electron microscopic appearance of filaments decorated with heavy meromyosin.
much greater than those at the pointed end of the filament. With the use of barbed-end blocking proteins, however, it would seem possible to block all events at the barbed end and thus obtain the rate constants and other characteristics of the pointed end of the filaments. By subtraction of these values from the values already available for the two ends combined, the rate constants for the barbed end alone could then be calculated.

Plasma gelsolin (also called brevin) is a barbed-end capping protein (12-18), M, 93,000, that is closely related to cytoplasmic gelsolin (19-29), M, 90,000. Plasma gelsolin has an additional 25 amino acids at the NH₂-terminal end (23) but is similar to cytoplasmic gelsolin by peptide mapping and immunochemical analysis (18, 25, 27, 28). Both proteins have been shown to bind to monomeric actin and to actin filaments, to nucleate actin assembly, and to increase the concentration of unphosphorylated actin at steady state. But reports differ on the extent of Ca²⁺ dependence of the binding of plasma gelsolin to G- and F-actin (14), and only one paper has been published in which plasma gelsolin was used to obtain the association and dissociation rate constants for ATP-G-actin at the pointed ends of actin filaments (15). We have now reinvestigated in greater detail the interactions of plasma gelsolin with G- and F-actin and determined the Ca²⁺ dependence of both reactions. We have also used the property of plasma gelsolin to cap the barbed end of actin filaments to try to get additional information on the kinetic parameters of the pointed end. The results of the latter experiments turned out to be more ambiguous than anticipated, suggesting that there is still much to be learned about either the reaction of gelsolin with actin, the properties of the pointed end of actin filaments, or both.

MATERIALS AND METHODS

Rabbit muscle G-actin was prepared by the usual methods (30, 31) followed by gel filtration on Sephadex G-200 equilibrated in buffer G consisting of 5 mM Tris-HCl, pH 7.8, 0.2 mM diithiothreitol, 0.1 mM CaCl₂, 0.2 mM ATP, and 0.1% NaN₃. Monomeric actin was stored on ice, and its concentration was determined from its absorbance at 290 nm using an extinction coefficient of 0.617 mg⁻¹ ml cm⁻¹ (32).

**Fig. 1. Fluorescence enhancement of NBD-G-actin upon binding to gelsolin.** NBD-G-actin and gelsolin were mixed in buffer containing 5 mM Tris-Cl, pH 7.8, 0.1 mM CaCl₂, 0.2 mM diithiothreitol, 0.2 mM ATP, 0.1 mM MgCl₂, and 0.1% NaN₃. Then successive additions of 0.3 mM EGTA (C), 0.3 mM CaCl₂ (D), and 0.8 mM EGTA (E) were made to each tube. The samples were incubated for 60 min at 25 °C after each addition and their fluorescence intensities measured. The fluorescence of NBD-G-actin alone was subtracted from the fluorescence intensity of each sample. A, the gelsolin concentration was constant at 50 nM. B, the NBD-G-actin concentration was constant at 0.2 μM.

Binding of Gelsolin to G-actin—The interaction of gelsolin with G-actin was measured by the enhancement of fluorescence of NBD-actin below its critical concentration in buffer G containing 0.1 mM MgCl₂. Fig. 1 shows the results obtained when increasing amounts of NBD-G-actin were added to 50 nM gelsolin (panel A) and when increasing amounts of gelsolin were added to 0.2 μM NBD-G-actin (panel B). No

**The abbreviations used are:** pyrenyl, N-pyrenyl carboxyamido-methyl; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; GA₂₀, 1:2 molar complex of gelsolin and actin; NBD, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole.
increase in fluorescence occurred when the two proteins were mixed in the presence of 0.3 mM EGTA and 0.1 mM CaCl₂ (Fig. 1, ○). When more CaCl₂ was added to the same samples to a final concentration of 0.5 mM (Fig. 1, △), the fluorescence increased linearly with the actin (A) or gelsolin (B) concentration until the actin-gelsolin molar ratio became 2 and then remained constant thereafter. These results indicate stoichiometric binding of actin to gelsolin, in the presence of Ca²⁺, forming a 1:2 gelsolin-actin complex (GA₂). The affinity of gelsolin for actin is too high to be calculated accurately from these data but the $K_D$ is apparently less than 10 nM under these conditions. As noted previously by others for platelet gelsolin (26), the fluorescence intensity of NBD-actin in GA₂ was about the same as in F-actin (twice the fluorescence intensity of NBD-G-actin) whereas the fluorescence intensity of pyrenyl-actin in GA₂ was only about 1.5-fold greater than that of pyrenyl-G-actin (data not shown and Refs. 15 and 16), in contrast to the 25-fold greater fluorescence intensity of pyrenyl-F-actin.

When excess EGTA (0.8 mM) was added to the same samples (Fig. 1, ○), the fluorescence intensity decreased by 50% in all cases. In order to determine if this decrease in fluorescence intensity was due to a lower quantum yield of the NBD-actin fluorescence in the GA₂ complex in EGTA or to partial dissociation of the complex upon addition of EGTA, similar samples (higher protein concentrations but in the same ratio and in the same buffers) were subjected to sedimentation velocity and sedimentation equilibrium analyses. As expected from the fluorescence data, when mixed in the presence of EGTA, NBD-G-actin and gelsolin did not interact; all of the actin remained as monomers of $M_r$, 45,000 with a sedimentation coefficient of 3 S (data not shown). In the presence of excess CaCl₂ all of the NBD-actin was bound to gelsolin as GA₂ and only one actin-containing species, of $M_r$, 180,000 (Fig. 2, ○) and sedimentation coefficient 8 S (data not shown), was detected, even in the presence of excess gelsolin. When excess EGTA was added to the solution containing the GA₂ complex, the plot of $\ln$ (absorbance) versus (radius)$^2$ became curved (Fig. 2, ○). The slopes of the tangents at the extremes corresponding to the bottom of the cell and the meniscus indicated the presence of species of $M_r$ 120,000 and 45,000, respectively. Therefore, addition of excess EGTA

![Figure 2](image)

**FIG. 2.** Sedimentation equilibrium of the gelsolin-actin complex in the presence of CaCl₂ (○) or EGTA (△). NBD-actin (19 μM) was incubated with gelsolin (10 μM) for 30 min at 25 °C in buffer containing 5 mM Tris-HCl, pH 7.8, 0.1 mM CaCl₂, 0.1 mM MgCl₂, 0.2 mM dithiothreitol, 0.2 mM ATP, and 0.01% NaN₃. The sample was then divided into two aliquots with one aliquot adjusted to 0.5 mM EGTA. After another hour of incubation 100 μl of the two samples were loaded into the cells and centrifuged at 12,000 rpm. The concentration of protein as a function of radial distance from the center of rotation was determined by scanning the cell at 480 nm, the absorption peak of NBD-actin.

![Figure 3](image)

**FIG. 3.** Effect of gelsolin on the rate of actin polymerization in the presence of Ca²⁺. A, the polymerization of 25 μM actin (5% pyrenyl-labeled) in buffer G was induced by 1 mM MgCl₂ in the absence (○) or in the presence of 0.5 nM (○), 1.5 nM (△), 3 nM (△), 5 nM (●), 8 nM (■), 11 nM (○), 22 nM (●), 51 nM (○), 100 nM (■), or 155 nM (○) gelsolin. B, for each assay the observed rate constant was obtained from the slope of the linear portion of the semilog plot of the change of fluorescence ($F_w - F_t$) versus time. In each assay, $F_w$ (the fluorescence at steady state) was determined 3 h after initiating polymerization. The values were: 9.75 (○), 9.72 (○), 9.69 (△), 9.78 (△), 9.58 (●), 9.78 (■), 7.5 (○), 7.5 (●), 7.5 (■), 7.5 (○), and 7.2 (○).
to G\(\alpha_2\) in Ca\(^{2+}\) dissociated the G\(\alpha_2\) complex into a 1:1 gelsolin-actin complex and free G-actin monomer.

**Effects of Gelsolin and G\(\alpha_2\) on Actin Polymerization**—All of the remaining experiments were done with pyrenyl-actin rather than NBD-actin so that the presence of G\(\alpha_2\) would not affect the quantification of F-actin. In the presence of Ca\(^{2+}\), both gelsolin (Fig. 3A) and G\(\alpha_2\) (Fig. 4A) decreased, but did not eliminate, the lag phase in the polymerization of actin, and both increased the rate of actin polymerization. The remaining lag probably reflected the time required for the exchange of G-actin-bound Ca\(^{2+}\) by Mg\(^{2+}\) (38, 39) but, as will be discussed later, may be due in part to the fact that G\(\alpha_2\) itself is probably not a true nucleus.

Figs. 3B and 4B are plots of the observed polymerization rate constant, \(k_{\text{obs}}\) (which is the product of the association rate constant, \(k_a\), and the concentration of growing filament ends) as a function of the gelsolin or G\(\alpha_2\) concentration calculated from the data, after the lag phases, in Figs. 3A and 4A. At very low concentrations (i.e., at approximately the concentration of filaments that would have occurred in the absence of capping proteins), gelsolin, and probably also G\(\alpha_2\), caused a decrease in \(k_{\text{obs}}\). Then, between molar ratios of gelsolin or G\(\alpha_2\) to actin of about 1:1200 and 1:150, \(k_{\text{obs}}\) increased linearly. At yet higher ratios, the increase in \(k_{\text{obs}}\) was no longer proportional to the concentration of gelsolin or G\(\alpha_2\).

The initial decrease in \(k_{\text{obs}}\) can probably be explained by the capping of the more rapidly growing barbed ends of filaments which would then be able to elongate only at the slowly growing pointed ends. At higher concentrations of gelsolin and G\(\alpha_2\), sufficiently more nuclei would be created to compensate for the loss of barbed-end growth, and \(k_{\text{obs}}\) would increase in proportion to the increase in filament concentration caused by the capping protein. During this phase, \(k_{\text{obs}}\) would be expected to be the product of the filament concentration and the association rate constant for ATP-G-actin at the pointed end, \(k_a^{2+}\text{ATP}\). Therefore, if the filament concentration were assumed to be equal to the gelsolin and G\(\alpha_2\) concentrations, \(k_a^{2+}\text{ATP}\) could be calculated. We will discuss this later.

In the presence of excess EGTA, the effects of gelsolin on actin assembly were very different (Fig. 5); gelsolin had no effect on the lag phase and decreased slightly the rate of elongation but in a complicated way. The rate of elongation progressively decreased at ratios of gelsolin to actin between 1:180 and 1:20 (80 nm–0.7 \(\mu\)M gelsolin) and then increased at ratios between 1:10 and 1:3 (1.8–5.5 \(\mu\)M gelsolin). The inhibition phase probably reflects a very low affinity of gelsolin for barbed ends in EGTA, and the slight reversal of this inhibition at very high gelsolin concentrations may reflect a very slight filament-severing activity of gelsolin in EGTA. The reversal of inhibition cannot be due to nucleation because plasma gelsolin does not form a complex with G-actin in EGTA (Fig. 1). More data on these two issues will be presented later.

**Effect of Gelsolin on the F-actin Steady State**—Different concentrations of gelsolin were added to solutions of pyrenyl-G-actin in the presence of 1 mM MgCl\(_2\) and 0.1 mM CaCl\(_2\) with or without 0.3 mM EGTA and the fluorescence intensities measured after 4 h at 25 °C, by which time steady state had been reached. The experimental data in Fig. 6 are plotted as the concentration of nonfluorescent actin, i.e., actin that had the fluorescence of pyrenyl-G-actin (and pyrenyl-actin in G\(\alpha_2\)) in the presence of EGTA. Solutions of 15 \(\mu\)M actin (5% pyrenyl labeled) in buffer G, pH 7.8, were incubated with 0.05 mM MgCl\(_2\) and 0.3 mM EGTA for 3 min before initiation of polymerization. At zero time MgCl\(_2\) was added to a final concentration of 1 mM, and various amounts of gelsolin were added: 0 nM (O), 4 nM (A), 80 nM (A), 0.4 \(\mu\)M (E), 0.7 \(\mu\)M (D), 1.8 \(\mu\)M (C), 3.2 \(\mu\)M (F), 5.5 \(\mu\)M (G). The steady state fluorescence was read 4 h after the polymerization was initiated.
of the critical concentration of ATP-actin at the pointed end and the concentration of GA₂ (as the complex or at the barbed ends). We believe that point is represented by the brief plateau that occurred around 50 nM gelsolin. We have no explanation for the overshoot that reproducibly occurred at lower gelsolin concentrations.

The dashed line in Fig. 6 is the expected concentration of GA₂ (both as the 1:2 complex and at barbed ends) which will increase in direct proportion to the concentration of gelsolin until all of the actin exists as GA₂ (8 µM gelsolin for the 16 µM actin used in this experiment). The concentration of nonfluorescent actin represented by the difference between the experimental curve and the theoretical curve for GA₂ will be the concentration of monomeric G-actin, i.e. the true critical concentration for ATP-actin at the pointed end. This value remained constant at 4 µM between about 0.1 and 6 µM gelsolin, after which actin monomer was converted to GA₂ complex until, at 8 µM gelsolin, the experimental and theoretical curves met.

In the presence of EGTA, the critical concentration of actin in the absence of gelsolin was 0.15 µM. The lower critical concentration for actin in the presence of EGTA is due partially to the increase in ionic strength but mostly to the removal of Ca²⁺ (41). With increasing amounts of gelsolin the concentration of nonfluorescent actin increased until it reached a plateau at 4.5 µM (i.e. about the critical concentration of the pointed end) at about 0.5 µM gelsolin and then remained constant as the gelsolin concentration was increased to 5.5 µM (the total actin concentration was 10 µM in this experiment). These data confirm the findings that, in EGTA, plasma gelsolin does not form a GA₂ complex (Fig. 1) but does bind weakly to the barbed ends of filaments (Fig. 5). One measure of the relative affinities of plasma gelsolin for barbed ends in the presence of Ca²⁺ and EGTA is that the pointed end critical concentration was reached at 20–40 nM gelsolin in Ca²⁺ while 0.5 µM gelsolin was required in EGTA.

**Effects of Gelsolin and GA₂ on the Rate of Depolymerization of Actin Filaments**—In the experiment described in Fig. 7, 20 µM actin was polymerized and then the F-actin was diluted 16-fold into solutions containing increasing concentrations of GA₂. As this diluted the concentration of actin greatly below its critical concentration, the filaments depolymerized. The rate of depolymerization was monitored by the decrease in fluorescence of pyrenyl-actin. Depolymerization was inhibited by the presence of GA₂. When the initial rate of depolymerization (where it is reasonable to assume that the filament concentration was the same in all samples) was plotted as a function of GA₂ concentration (Fig. 7A), it was readily seen that the depolymerization rate decreased to a plateau value, in this experiment, which was about 10% of the value obtained in the absence of GA₂.
The rate of depolymerization in the absence of GA₂ is the sum of the rates of depolymerization at both ends.

\[ V_o = V_p + V_b = k_p N + k_b N \]  \hspace{1cm} (3)

Assuming that GA₂ blocks all association and dissociation reactions at the barbed ends without affecting pointed-end events, the variation of the depolymerization rate as a function of GA₂ concentration will be,

\[ V = V_o - V_p = V_o - V_b = \frac{1}{1 + K(GA_2)} + V_p \]  \hspace{1cm} (4)

where \( K \) is the equilibrium association constant of GA₂ with the barbed end. In the absence of GA₂, \( V = V_o = V_p + V_b \) whereas, at infinite concentration of GA₂, \( V = V_o = V_p \). Rearrangement of Equation 4 leads to Equations 5 and 6, which are the Lineweaver-Burk and Dixon representations, respectively.

\[ \frac{1}{V_o - V} = \frac{1}{V_o - V_o (1 + K(GA_2))} \]  \hspace{1cm} (5)

\[ \frac{V_o - V_o}{V - V_o} = 1 + K(GA_2) \]  \hspace{1cm} (6)

When the data in Fig. 7A were plotted according to Equation 5 (Fig. 7B) and Equation 6 (Fig. 7C), we obtained a \( K_D \) of 19–22 nm for the interaction of GA₂ with barbed ends and a \( V_o \) equal to 3.4% of \( V_o \). This extrapolated value for \( V_o \), the rate of depolymerization at the pointed ends, was never reached experimentally, even with concentrations of GA₂ more than 10 times the \( K_D \) where complete saturation should have occurred. In four experiments, the rate of depolymerization at saturating concentrations of GA₂ plateaued at 6–10% of the value obtained in the absence of GA₂, i.e. \( k_b \) was 6–10% of \( k_b + k_b \).

Very different results were obtained when the filaments were diluted into solutions containing gelsolin instead of GA₂. In the presence of Ca²⁺, a rapid depolymerization occurred within seconds followed by a much slower depolymerization but one that still was faster than depolymerization in the absence of gelsolin (Fig. 8A). The rapid phase of depolymerization probably was the result of severing of filaments by gelsolin producing a very much greater concentration of filaments; thus, depolymerization would be faster than in the absence of gelsolin even though the barbed ends were capped. On the other hand, when actin filaments were diluted in the presence of EGTA, gelsolin had no effect on the rate of depolymerization at concentrations as high as 220 nM (Fig. 8B), confirming previous evidence that gelsolin binds only very weakly to barbed ends in the absence of Ca²⁺. The rate of filament depolymerization was accelerated at very high concentrations of gelsolin in EGTA (2.2 µM gelsolin versus 0.5 µM actin), perhaps reflecting a very weak filament-severing action of gelsolin in EGTA.

**Filament Growth at the Pointed End as a Function of Actin Monomer Concentration**—In an attempt to get more information about the association and dissociation rate constants at the pointed end of actin filaments, the initial rate of growth of actin filaments capped at their barbed ends by plasma gelsolin was determined as a function of actin concentration (Fig. 9). Such a plot has a positive and negative branch which correspond, respectively, to the lengthening and shortening of the filaments above and below their critical concentration (9). In the case of a simple equilibrium polymer, a straight line would be obtained with a slope of \( (k_b + k_p)N \), an intercept on the y axis of \( -(k_b + k_p)N \), and an intercept on the line of zero rate of c.
Three separate experiments were done using three different seed solutions of polymerized actin. Each seed solution contained the same concentration of gelsolin (19 nM, final concentration) but different concentrations of actin; the gelsolin:actin ratios in the seed solutions were 1:2 (i.e. the GA2 complex), 1:50, and 1:135 (i.e. short barbed-end capped filaments). Nonlinear plots, above and below the critical concentration, were obtained in each case with the rate of growth increasing nonlinearly with the actin monomer concentration up to about 10 μM. Above 10 μM actin, all of the curves appear to become linear when corrected for the polymerization due to spontaneous nucleation. This correction was never greater than 25%. It should also be noted that, although the gelsolin concentration, and hence the presumptive filament-seed concentration, was the same in all three experiments the rates of growth at every actin monomer concentration in the positive branch of the plot were greater in proportion to the ratio of acting gelsolin in the seeds. On the other hand, the actin concentration at which neither positive or negative growth occurred, i.e. the presumptive critical concentration for the pointed end, was always about 4 μM. This kinetic value for the critical concentration is in good agreement with the critical concentration determined in the steady state experiments (Fig. 6). As expected, the GA2 seeds did not depolymerize below the critical concentration.

To test the initial assumption that the filament concentration in each sample in Fig. 9 was constant and equal to the concentration of added seeds, G-actin solutions at concentrations between 5 and 20 μM were seeded with either GA2 or gelsolin-F-actin complex (1:50) and allowed to polymerize to steady state. Aliquots were then diluted 8-fold and the initial rates of depolymerization (k_{d}^{p}N) measured. If the filament number concentration had been determined only by the concentration of seeds, the initial rates of depolymerization would have been the same in all samples within each set. Instead, the depolymerization rates increased with the actin monomer concentration, although only slightly above 15 μM (Table I). The simplest interpretation of these data is that the filament number concentration increased with the monomer concentration up to about 15–20 μM actin.

**DISCUSSION**

The data in Figs. 1 and 2 confirm (15, 16) that plasma gelsolin forms a very tight 1:2 complex with G-actin in the presence of Ca^{2+}, with no 1:1 complex being detectable by either the fluorescence assay or by analytical ultracentrifugation, and show that no interaction between plasma gelsolin and G-actin occurs in the presence of excess EGTA. These results are in complete agreement with previous observations for cytoplasmic gelsolin (26). We have also found that addition of EGTA to the GA2 complex causes it to dissociate into a 1:1 complex that is stable for at least 3 days with no evidence for the existence of a 1:2 complex in EGTA. The apparent thermodynamic inconsistency between the ability to form a 1:1 complex in EGTA from the 1:2 complex but not from the free monomers suggests that either 1) the rate of dissociation or formation of the 1:1 complex in the absence of Ca^{2+} is very slow, in which case we do not know whether the 1:1 complex is or is not the thermodynamically stable state in the absence of Ca^{2+}, or 2) EGTA removes gelsolin-bound Ca^{2+} much more slowly from the 1:1 complex than from the monomer. The latter possibility seems more likely as platelet gelsolin in the 1:1 complex has been shown to retain 1 Ca^{2+} in the presence of EGTA (26). We find that both molecules of NBD-actin in the GA2 complex with plasma gelsolin have the same fluorescence as NBD-actin in F-actin. This latter observation is different from the report (26) that the first of the two NBD-actin molecules that bind to cytoplasmic gelsolin retains the fluorescence of NBD-G-actin in GA2.

The data in Figs. 3, 4, 6, and 7 provide extensive quantitative evidence that plasma gelsolin caps the barbed ends of filaments, and that plasma gelsolin binds very weakly to the barbed ends of filaments and even more weakly, if at all, sever actin filaments. Again these results agree with those for cytoplasmic gelsolin (19) but differ significantly from a previous report that plasma gelsolin binds to filament barbed ends almost as well in EGTA as in Ca^{2+} (14).

What have we been able to learn about the kinetic characteristics of the pointed end of actin filaments from these experiments? The data in Figs. 6 and 9 are consistent with a critical concentration for ATP-actin at the pointed end of 4 μM in buffer G containing 1 mM Mg^{2+}. This compares to values for the barbed end of about 0.35 μM for ATP-capped filaments (40) and 3 and 8 μM for both the barbed and pointed ends of ATP-actin filaments and ADP-actin filaments at equilibrium with ATP-G-actin (10, 11) and ADP-G-actin (9), respectively, in the same buffer. The ratio of pointed and barbed-end critical concentrations is similar to the ratios found by others under different ionic conditions with different barbed-end-capping proteins (43, 44).

From the data in Fig. 7, obtained with GA2, which caps barbed ends but does not sever filaments and thereby does not change the filament concentration, we calculated that the dissociation rate constant for ADP-actin from the pointed end of a filament is on average about 6% of the dissociation rate constant for ADP-actin from both ends of an uncapped filament. As this latter value has been determined to be about 6.4 s^{-1} in the same buffer (42), k_{d}^{ADP}, the dissociation rate constant for ADP-actin at the pointed end, is probably 0.4 s^{-1}. It should be noted that this is the k_{d} for ADP-actin, not ATP-actin, because even if an ATP cap were present at the pointed end at steady state (which is unlikely given that the critical concentration is more than 10 times greater at the pointed end than at the barbed end) it would have been lost rapidly during depolymerization exposing the ADP-actin core. The value of 0.4 s^{-1} can be compared to values of about 6 s^{-1} for k_{d}^{ADP}, the rate constant for the loss of ADP-actin from the barbed end of an ADP-actin filament, and about 0.6 s^{-1}
for $k^\text{ATP}_a$, the rate constant for dissociation of ATP-actin from the barbed end of an ATP-capped filament (45). The critical concentration is the ratio of $k_-/k_+$. Therefore, from the value of $k^\text{ATP}_a = 8 \mu M$ and critical concentration of ADP-actin = 8 $\mu M$ (for capped filaments (9, 42) and also for barbed-end capped filaments (data not shown)), the calculated value of $k^\text{ATP}_a$ = $5 \times 10^4 M^{-1} s^{-1}$. This can be compared to a value of $k^\text{ATP}_a = 7.5 \times 10^4 M^{-1} s^{-1}$ (42).

From the data in this paper, there are several different ways to evaluate the association rate constant for the pointed end in ATP, and, from this value and the critical concentration, the dissociation rate constant for the pointed end in ATP. In principle, we should be able to calculate $k^\text{ATP}_a$ from the linear portions of the plots of $k_{obs}$ versus gelsolin or GA$_2$ concentration in Figs. 3B and 4B. If we assume that each molecule of gelsolin or GA$_2$ had been a nucleus for filament growth, the pointed end concentration in those experiments would have been equal to the gelsolin or GA$_2$ concentration and simply dividing $k_{obs}$ by that concentration would give $k^\text{ATP}_a$. By this calculation, values for $k^\text{ATP}_a$ of 2.2 $\times 10^4 M^{-1} s^{-1}$ and $8 \times 10^4 M^{-1} s^{-1}$ are obtained from Figs. 4B and 3B, respectively. These values can be compared to the value of $5 \times 10^4 M^{-1} s^{-1}$ obtained by Doi and Frieden (15) in 1.5 mM MgCl$_2$ with 5.9 $\mu M$ G-actin and 59 mM plasma gelsolin. However, the $k_{obs}$ values in Figs. 3B and 4B were obtained at different single actin concentrations, and it can be seen from Fig. 9 that $k_{obs}$ varied with the actin concentration. This actin-concentration dependence of $k_{obs}$ was also noted by Doi and Frieden (15), but they did not consider it when calculating $k^\text{ATP}_a$. The data in Fig. 9 and Table I also strongly suggest, as explained below, that the filament concentration was not equal to the gelsolin concentration at all actin concentrations. If this is correct, the above calculation of the association rate constant might not be valid, and a dissociation rate constant calculated from that value and the critical concentration might also be in error.

Why do the data in Fig. 9 indicate that the filament concentration was not equal to the gelsolin concentration? Irrespective of the detailed polymerization model, the rate of depolymerization extrapolated to zero actin concentration, i.e. the intercept on the y axis (Equation 2) will be $-(k^\text{ADP}_a + k^\text{ADP}_N)/k_+$ or, for barbed-end capped filaments, simply $-(k^\text{ADP}_a)/k_+$ (9, 10). In Fig. 9, this value is about 0.8-1.25 $10^5 M^{-1} s^{-1}$ for the experiments in which seeds were prepared with gelsolin/actin ratios of 1:50 and 1:135. (The experiment with GA$_2$ as seeds cannot be used to determine $k^\text{ATP}_a$ because GA$_2$ cannot depolymerize.) When the depolymerization rate of 0.8-1.25 $10^5 M^{-1} s^{-1}$ is divided by $k^\text{ADP}_a = 0.4 s^{-1}$, the value determined from Fig. 7, a filament concentration of about 2-3 $nM$ is calculated. This is only 10-15% of the concentration of gelsolin in 19 nM used in the experiments to obtain the data in Fig. 9. Therefore, the filament concentration must have been much less than the gelsolin concentration in the negative branches of the plots in Fig. 9.

Moreover, the fact that the plots are curvilinear in their positive branches until about 10 $\mu M$ actin strongly suggests that the number concentration of growing filaments increased with actin concentration at least until that point. This would explain why $k_{obs}$ and, therefore, the calculated $k^\text{ATP}_a$ was greater in Fig. 3B (actin concentration, 15 $\mu M$) than in Fig. 4B (actin concentration, 5 $\mu M$). It is possible that the presence of an ATP cap at the pointed end also contributed to the curvilinear plot (9), although for the barbed end this effect is seen only very near and below the critical concentration (9, 10). Above 10 $\mu M$ actin, the plots become linear indicating, within experimental error, that the filament concentrations were approximately constant in that region, especially at the higher ratios of gelsolin:actin (Table I).

The slope at any point in the upper branches of the curves in Fig. 9 will be $k^\text{ATP}_a$. N. On the assumption that N, the filament concentration, is equal to the gelsolin concentration when the curves are linear, the calculated value for $k^\text{ATP}_a$ is 0.78-0.92 $10^5 M^{-1} s^{-1}$ for the three curves. Therefore, it seems likely that a reasonable approximation for $k^\text{ATP}_a$, calculated from the depolymerization experiment described in Fig. 7, is about 1 $10^5 M^{-1} s^{-1}$. If we multiply the critical concentration, 4 $10^4 M$, by this value the calculated $k^\text{ATP}_a$ at steady state would be 0.4 $s^{-1}$ which is the same as the value calculated for $k^\text{ADP}_a$ from the depolymerization experiment described in Fig. 7. This suggests that the pointed end may not have an ATP cap at steady state. Doi and Frieden (15) estimated $k^\text{ATP}_a$ to be only 0.05-0.1 s$^{-1}$ from a similar calculation, but they used a value for $k^\text{ATP}_a$ which we believe to be too low because it was calculated from rate measurements made at only 5.9 $\mu M$ actin.

All of the kinetic and steady state rate constants determined in this laboratory for the pointed and barbed ends of ADP-actin and ATP-actin in 1 mM MgCl$_2$ and 0.2 mM ATP are summarized in Table II.

The conclusion that, at least at low actin concentrations, the concentration of filaments was very much less than the concentration of gelsolin used to nucleate filament growth means that much of the gelsolin must still have been present as GA$_2$. If GA$_2$ were always present, the filament concentration would continuously increase as the actin monomer concentration was increased by nucleation of new filaments from GA$_2$. This is equivalent to saying that GA$_2$ is not a true nucleus for actin polymerization, i.e. the affinity of ATP-G-actin for GA$_2$ may be much less than its affinity for a filament end. This conclusion is reinforced by the data in Fig. 6. According to the linear polymerization model for short filaments (46), when the concentration of free monomer should decrease proportionately as filaments become shorter. In the experiment described in Fig. 6, the monomer concentration should, therefore, have decreased linearly from the value of 4 $\mu M$ at 20 nM gelsolin (when all of the barbed ends were capped) to 2 $\mu M$ at 4 $\mu M$ gelsolin and 1 $\mu M$ at 6 $\mu M$ gelsolin. Instead, the monomer concentration remained constant at 4 $\mu M$ throughout this range suggesting that relatively long barbed-end capped filaments were in equilibrium with monomer and GA$_2$.

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